"Primitive Knot and Early Gastrulation Cavity co-existing with Independent Primitive Streak in *Ornithorhynchus*."

By Professor J. T. Wilson, M.D., and J. P. Hill, D.Sc., University of Sydney. Communicated by Professor G. B. Howes F.R.S.

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Amongst the material at our disposal for the investigation of the earlier stages in the development of the Monotremes is an egg of Ornithorhynchus, measuring 10 × 9·5 mm. The stage of development represented by this egg is of such interest and importance that we have deemed it deserving of a brief preliminary communication.

The stage of embryonic organisation would appear to fit in as immediately succeeding the oldest of the early embryos described by Semon,* viz., his "E_6" (fig. 15, Taf. 8). His "E_7" appears, indeed, to have been older than "E_6," but with the exception of one figure (fig. 39, Taf. 9) representing the structure of the extra-embryonic blastoderm, Semon gives no indication of the conditions met with in that ovum. This is the more to be regretted, as we see reason to believe that "E_7" must have corresponded pretty closely with the stage now to be described. Semon notes that the embryonic area of this egg was injured through an unfortunate accident.

The general structural arrangements of the Monotreme ovum in its early stages of development have been described and figured by Semon. In all the stages dealt with by him, illustrating the development from segmentation up to the first indications of gastrulation, the yolk had retained its original arrangement as a tolerably coherent solid or semi-solid spheroidal mass with alternating strata of white and yellow yolk-spheres. As he states, however, the egg in the course of further development increases considerably in size during its sojourn in the uterus through absorption of fluid. By this process of fluid absorption, the yolk-mass is disintegrated and its spherules disseminated throughout the interior of the growing blastodermic vesicle, though many of them remain adherent to the deep surface of the blastodermic membrane.

This conversion of the solid or semi-solid yolk-mass into the fluid contents of a large blastodermic vesicle renders the investigation of the structure of the blastoderm, from the period of the commencement of gastrulation up to the formation of a distinct embryo, an exceedingly difficult one. The loss of Semon's "E_7" may very likely be attributable to such difficulties in the way of manipulation as we have encountered in dealing with a delicate blastodermic membrane surrounded by a thick, tough, and opaque shell as well as a vitelline

* Semon, R., 'Zool. Forschungsreisen,' &c., Bd. 2, Lief. 1 (1894).

membrane, and distended by a considerable bulk of fluid material. It is quite impossible to remove the shell without serious damage to the delicate blastoderm. Fixation with the shell intact is imperative, and, as a matter of fact, the result in the way of preservation proves quite satisfactory, as evidenced by the condition of the cellular blastoderm, in which mitotic figures are well preserved. But even after fixation, the opening up of the ovum is attended with no little risk. The inevitable evacuation of the contained fluid allows of crumpling of the blastodermic membrane with possibility of injury to the embryonic area. It is impossible, owing to its size and osmotic difficulties, to treat the ovum throughout unopened. Even were that course practicable, the impossibility of orientation would be an insuperable difficulty.

After fixation and subsequent dehydration in graded alcohols, the 10 mm. egg was cleared in origanum oil and opened. The blastoderm still remaining *in situ* in relation to the shell was examined from the interior aspect, and was found to possess at one spot a small more opaque area, somewhat oblong, but rather irregular. The portion of the blastoderm containing this small opaque patch was photographed by transmitted light at a magnification of 6·5 diameters for the purpose of orientation.

Our surmise that the area in question was of the nature of an embryonic or primitive knot was afterwards confirmed by the examination of serial sections.

No differentiation in way of an embryonic area in the wider sense is recognisable in the photograph, nor was any such discovered in the course of examination of the wall of the blastodermic vesicle *in toto* under low magnification. We naturally concluded that the very evident knot represented the earliest and only differentiated area, and for a time devoted our attention solely to this area and the blastoderm in its vicinity. The portion of the blastoderm containing the knot was separated from the remainder of the wall of the vesicle, and was then imbedded and cut in serial sections. Examination of these did in fact show that in the neighbourhood of the knot, and for some distance from it, the wall of the vesicle was destitute of any indication of further differentiation. But towards the periphery of the portion sectioned and comparatively remote (nearly 2 mm.) from the knot, itself, we found the commencement of a region of thickened ectoderm with underlying mesoderm. Our attention thus being directed to other manifestations of developmental activity in the blastoderm in addition to the primitive knot, we found, in the portion of the vesicular wall originally put aside, a quite extensive area showing important changes. These amount to no less than the establishment, quite away from the region of the knot, of a distinct linear primitive streak formation, surrounded by an area over which the ectodermal

layer is thickened and cubical, and within which a mesodermal sheet has already undergone a wide extension.

It is peculiarly unfortunate that, owing partly to the difficulty of dealing with the delicate and originally collapsed and torn vesicular wall after the cutting open of the egg, partly to the failure to detect any visible differentiation apart from the knot, and our consequent conviction that the latter was the sole trace of embryonic organisation yet present, the orientation of the distinct portions into which the vesicle was separated was not adequately determined and preserved. It thus became impossible for us to guarantee that the planes of sectioning of the remaining portions should be accurately co-ordinated with each other, or with that of the important piece first sectioned. This failure, not wholly blameworthy, when the conditions of the task are appreciated, has introduced an element of conjecture into our subsequent attempt to determine the precise relation to the knot of the primitive streak area, of whose existence we later on became aware. Nevertheless, we think that we shall be able to establish these relations with at least a high degree of probability.

In this preliminary paper we propose to restrict ourselves to an account of the highly interesting area which first attracted our attention, and which we have already referred to as a "primitive knot." It will, we think, be admitted that no possible doubt can be entertained of the justice of employing for its designation a term which would stamp it as the homologue of the well-known structure in many Sauropsida to which the same name has been applied. It is a true primitive or gastrula knot, in the Sauropsidan sense, possessing a transversely elongated gastrula-mouth or blastopore and an invagination cavity, which both in appearance and minute structure resembles in the closest manner the structure described under these names in various Reptilian forms.

But if this be admitted, we come face to face with this extraordinary fact that, in addition to this reptilian-like gastrula, there is to be found in Ornithorhynchus, quite distinct and even remote from this gastrula knot, a region in which there is being differentiated independently a primitive streak of quite ordinary and typical mammalian character.

The oblong portion of the wall of the vesicle containing the primitive knot near its centre, and measuring about 8 × 10 mm., was left adhering to the portion of the shell covering it. The direct observation and photography of the piece were thus confined to its deep aspect so as to avoid undue disturbance and injury. The whole piece was then double imbedded in photoxylin and paraffin and cut into serial sections 10 micra thick. The sections were stained in hæmatëin and eosin.

The plane of section chosen was approximately at right angles to

the larger diameter of the embryonic knot, and parallel to its presumable axis, on the supposition that a clear spot near one of its margins represented the position of the gastrula opening or blastopore, thus determining the true posterior margin of the knot.

Examination of the series did ultimately show that the sections were approximately longitudinal sections through the gastrula cavity.

The general character of the egg at the stage under consideration must be briefly referred to. At the period now dealt with, the formerly yolk-laden ovum has become transformed into a large blastodermic vesicle with fluid contents, amongst which are large numbers of dispersed yolk-spheres of the original yolk-mass. One can, without hesitation, homologise the interior of the vesicle with the subgerminal cavity of a Sauropsidan egg, extended so as to include by liquefaction the whole of the yolk itself. Ornithorhynchus indeed may be said to afford an actual demonstration of the transformation of a Sauropsidan subgerminal cavity, such as is figured in its first beginnings in Semon's figures 36 and 38 of his "O_3," into the cavity of a mammalian blastodermic vesicle, thus supporting Keibel's view of the correspondence of these cavities.

The establishment of the vesicular stage has been effected by the extension of the cellular blastoderm completely around the yolk, a condition representing a considerable advance on that existing in Semon's figures "E_5" and "O_3." He does not record the condition in this respect of his "E_6." He was also unable fully to determine the condition of "E_7" in this respect. We believe that the latter must have been very similar to our present stage, judging from the figure he gives of a portion of the extra-embryonic blastoderm.

Not only is the cellular wall of the blastodermic vesicle complete in our specimen, but it is already bilaminar throughout, and trilaminar over a not inconsiderable area. A complete layer of yolk-entoderm ("secondary" entoderm) has been differentiated lining the cavity, except under the small area of the primitive knot, with whose tissue it is continuous. The yolk-entoderm cells are large and swollen, being distended by yolk-spheres of various sizes and somewhat different staining capacities. Owing to their yolk-laden character their protoplasmic contents are relatively greatly reduced. The nuclei are, as a rule, large and vesicular. In the least successful sections the entoderm cells may be broken up and more difficult of recognition. We are firmly convinced that it is owing to the imperfection of the section shown in Semon's figure 39 from his "E_7" that such a careful observer has been unable to recognise the yolk-entoderm as such, and figures it as a zone of vacuolated coagulum next the surface of the yolk. The imperfect layer of angular-looking cells which he figures and regards as the entoderm in "E_7" is not really such, but forms an intermediate layer of mesodermal cells.

This layer of mesoderm is well represented in the stage under description. But it by no means extends throughout the whole extent of the blastodermic vesicle, nor is it met with at all at or in the immediate vicinity of the primitive knot. It is found most fully developed in and around the primitive streak area, and here it is continuous with the ectodermal thickening of the primitive streak (*i.e.*, paraxially), where it attains greater thickness and consists of several layers of cells. Outwards from the line of the primitive streak* it thins out gradually into a single layer, and further out becomes patchy and incomplete. It is throughout distinct and independent of the underlying yolk-entoderm, which can be followed through the whole wall of the vesicle. We have several preparations of small portions of the wall of the vesicle stained and mounted *in toto*, which give clear demonstration of the huge yolk-laden entoderm cells forming a continuous lining of the vesicle wall, precisely as figured by Hill and Martin† in a considerably later stage.

The ectoderm of the vesicle wall throughout the greater part of its extent forms a membrane consisting of very thin flattened cells closely applied to the vitelline membrane. This character it retains in the vicinity and over a portion of the primitive knot. But over the region already referred to as the primitive streak area, remote from (posterior to) the primitive knot and extending outwards so as to be practically co-extensive with the mesoderm of this area, the ectoderm shows a marked change in character. Here its cells are no longer flattened and squamous, but thickened and cubical, and here also their developmental activity is often betrayed by the existence of frequent mitotic figures. In the posterior part of the primitive streak thickening, the cubical ectoderm gradually becomes more attenuated, the mesoderm still continuing beneath it for some distance, even after the "extra-embryonic" character of the ectoderm is assumed.

We now come to the "primitive knot" itself, whose occurrence and characters it is the special object of this brief paper to record.

The oblong area of the blastoderm constituting the knot measured 0·42 mm. in one diameter (antero-posterior axis of gastrula) and 0·49 mm. in the other diameter (transverse). It is therefore comparable in general form with the area from an Echidna egg (E_6) figured by Semon in his fig. 15 (*loc. cit.*). Unfortunately Semon gives no clue to the magnification of this figure, which is on a different scale to the other illustrations. One cannot therefore compare the area of E_6

* The plane of section through the primitive streak area of the vesicle wall, though its orientation was determined in rather haphazard fashion in default of any visible guide, is obviously approximately transverse to the axis of the primitive streak, showing the characteristic bilateral symmetry of organisation of this important region.

† Hill and Martin, "On a Platypus Embryo from the Intra-uterine Egg," 'Proc. Linn. Soc., N.S.W.,' vol. 10, figs. 31—33.

in size with that now described. It is to be regretted that Semon's specimen was lost through accident, so that no further comparison can be instituted. There is, however, a general resemblance between fig. 15 of Semon's E_6 and his figs. 14 and 18 showing areas from other eggs of Echidna (E_5) and Ornithorhynchus (O_3). The dimensions of these can be calculated and are both found somewhat to exceed those of our primitive knot, in spite of their relatively younger ages. It is therefore difficult to say to what extent the areas referred to (of E_6 and E_5 and O_3) correspond to our primitive knot. We are, however, of opinion, that E_6, though probably younger, must have pretty closely corresponded, and there can be little doubt but that the centre of the other areas, at least, is later on developed into an actual primitive knot. The sectional figure of O_3 shown in fig. 38, interpreted by Semon as showing the commencement of gastrulation, may possibly bear this interpretation, but we cannot feel satisfied that the granular coagulum layer between the cellular layer and the surface of the yolk shown in this figure has been adequately interpreted, especially in view of our complete conviction that in fig. 39 the layer represented in somewhat similar fashion is beyond all doubt really yolk-entoderm.

We do not wish to be taken as suggesting that here in O_3 the coagulum represents differentiated yolk-entoderm. We are simply not convinced beyond all doubt that all the formed cell elements of this area in O_3 are disposed in the form of a superficial continuous membrane as shown in fig. 38, more especially in view of the totally different condition of the nearly related stage E_5, as illustrated in the sectional fig. 33, and of the condition described below in our own somewhat later stage in Ornithorhynchus.

The general form of the embryonic knot has already been noted. A schematic surface projection of its outline is shown in fig. 1. This

FIG. 1.

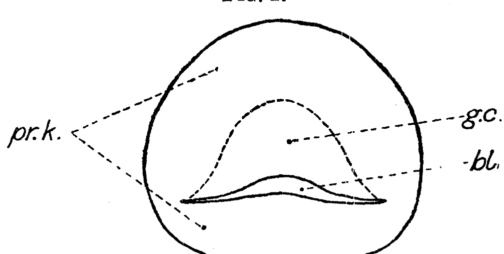
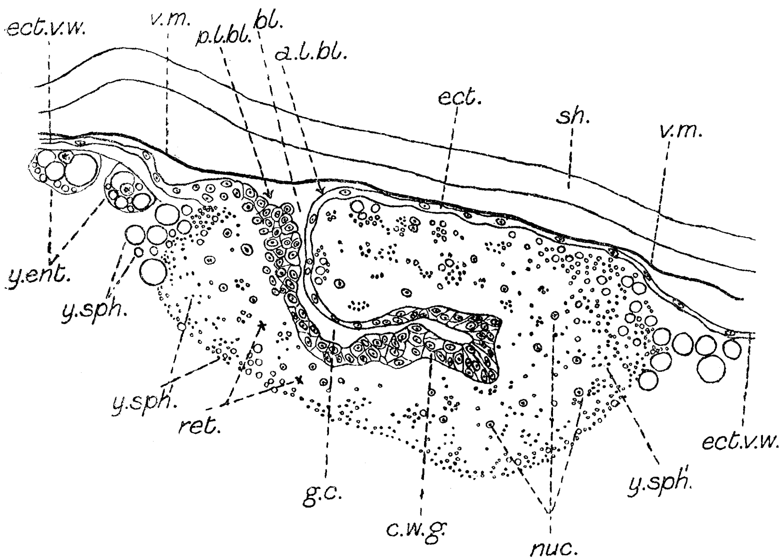


figure represents a plane reconstruction of the area of the knot, compiled from the serial antero-posterior sections through the region. One of the more typical of these sections is also figured semidiagra-

matically in fig. 2. More adequate illustration by photomicrography and otherwise will be forthcoming in a future communication.

FIG. 2.



In mesial section, the knot is seen to form a thick and prominent lenticular mass projecting into the cavity of the blastodermic vesicle. It is largely composed of a loose reticular tissue in which nuclei are only sparsely distributed and cell outlines are for the most part invisible. This tissue is thickly dotted with minute yolk-spherules and small vacuoles, and is not limited towards the cavity of the vesicle by any very sharp or clear-cut boundary. This reticulum of the knot is continuous peripherally with the yolk-entoderm of the bilaminar blastoderm around the knot.

Penetrating the interior of the knot is the archenteric or gastrula-cavity, opening on the surface at the blastoporic aperture near the hinder part of the knot and appearing in sagittal section as a curved canal passing from the blastopore at first deeply, and then forwards, to end blindly in the more anterior part of the knot. This cavity is lined throughout by a very definite cellular wall.

Both in front of and behind the knot, the blastoderm is simply bilaminar, with thin ectoderm closely applied to the deep surface of the vitelline membrane. The entodermal cells are large and contain yolk-spherules of varying size and staining reaction and loose yolk-spheres are also found adherent to its deep surface.

The thin ectoderm is continued over the knot from the region in

front without change of character as far as the transversely elongated blastopore seen in fig. 1 and in section in fig. 2. Thence it is continued round the anterior lip of the blastopore so as to line the roof of the blastoporic (archenteric) canal or gastrula-cavity.

Posteriorly to the knot the thin ectoderm is continued forwards, but on reaching the posterior edge of the knot, its cells lose their markedly flattened character, and become continuous with a mass of cuboidal or even rounded cells, which forms the prominence of the posterior lip of the blastopore. The superficial cells of this mass do not, however, wholly surrender their epithelial arrangement, and are continued forwards in the hinder wall and floor of the gastrula-cavity to complete the cellular lining of this cavity. This cellular lining consists, for the greater part of its extent, of large and cuboidal cells, but in the hinder part of the roof, and extending from this upwards around the anterior lip of the blastopore, the cells are more flattened, ultimately becoming continuous with the thin ectoderm overlying the anterior part of the knot.

Figs. 1 and 2 together enable one to realise the form of the gastrula-cavity as a wide, but dorso-ventrally flattened cavity opening posteriorly by a transversely-elongated blastoporic aperture.

The posterior lip of the blastopore is formed by a mass of cells which, if not wholly indifferent, at least show a less clear differentiation into superficial and deep than is found elsewhere.

As was indicated at the outset, the resemblance between the primitive knot in Ornithorhynchus and that found in a number of reptilian forms is very striking. We would particularly draw attention to the figures by Mitsukuri* of the primitive knot in Chelonia, and more especially to his figs. 9 and 13 on Plate 8, which show a remarkable similarity to the condition here described, in all essential features. In Ornithorhynchus we lack entirely the columnar arrangement of the ectoderm over the knot, and the anterior lip of the blastopore there shown, otherwise the characters both of the knot and of the gastrula-cavity and its wall appear to be almost identical with those figured, especially in Mitsukuri's fig. 9, Plate 8.

In a future more extended communication we hope to illustrate more adequately the points above set forth, and in addition to add something in the way of elucidation of the fate of the primitive knot in somewhat later stages. We propose also to describe and illustrate the condition of the primitive streak area, which at the period now dealt with already co-exists with, though independently of, the primitive knot, but which later by extension comes into more intimate relationship with the knot.

* K. Mitsukuri, "On the Process of Gastrulation in the Chelonia," 'Journ. Coll. Sci., Japan,' vol. 6. *Cf.* also Gertrude C. Davenport, 'Radcliffe Coll. Monograph,' No. 8. Boston, 1896. Dendy, 'Qu. Jl. Micro., Sci.,' vol. 42, p. 18, 1899.

The mere fact of its co-existence at this stage with the knot, necessarily occasions some reconsideration of the morphological relationship of the mammalian primitive streak to the process of gastrulation.

"The Brain of the Archæoceti."* By G. ELLIOT SMITH, M.A., M.D., Fellow of St. John's College, Cambridge, Professor of Anatomy, Egyptian Government School of Medicine, Cairo. Communicated by Professor G. B. HOWES, LL.D., D.Sc., F.R.S. Received January 15,—Read February 12, 1903.

So far as I have been able to ascertain, nothing whatever is known of the form of the brain or, more strictly, of the cranial cavity in the Archæoceti. Hence no apology is needed for presenting even this imperfect account of two cranial casts representative of this sub-order, which have come into my hands.

Among the Eocene remains found in the Fayûm region of the Egyptian desert by Mr. H. J. L. Beadnell and Dr. Charles W. Andrews, in 1901, there was a broken skull of *Zeuglodon*,† from which it was possible to obtain a mould, representing the form of the greater part of the dorsal and lateral aspects of the brain. A plaster cast was made in the British Museum at the instance of Dr. Andrews, who kindly placed it at my disposal for description.

In the following winter (1902), Mr. Beadnell found in the same locality a natural cranial cast of the same size and general form as the artificial cast of *Zeuglodon*. It is obvious at a glance, if the two specimens be placed side by side, that the natural mould belongs to some member of the Archæoceti, but whether to the same species or even genus as the other specimen must at present remain an open question.

Mr. Beadnell kindly placed this specimen at my disposal.

The size and relative proportions of the different parts are almost identical in the two casts. Nevertheless, there are a considerable number of differences, some features being displayed in one and not in the other, and *vice versâ*. Many of these differences are obviously due to the imperfections of the casts, and especially to the failure of the plaster mould to represent the true form of the brain. But there are

* These notes were originally intended for the Report on the Survey of the Fayûm, to be issued by the Egyptian Survey Department, and are now published separately with the permission of the Under Secretary of State for Public Works and Captain H. G. Lyons, Director-General of the Survey Department.

† C. W. Andrews, "Extinct Vertebrates from Egypt," Part II. (Extracted from the 'Geological Magazine,' N.S., Decade IV, vol. 8, 1901, p. 437,—*Zeuglodon Osiris*, Dames'.)